cDNA Cloning and Nucleotide Sequences of α_1 and α_2 Thionins from Hexaploid Wheat Endosperm

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Thionins are homologous Cys-rich proteins of about 5 kD that have been isolated from different tissues in a wide range of plant taxa and are active against plant pathogens both in vitro and in vivo (Fernández de Caleya et al., 1972; García-Olmedo et al., 1992; Carmona et al., 1993). The available amino acid sequences (either directly determined or deduced from cDNAs) have been classified into five well-defined structural types. Two of these types, I and V, are present in wheat endosperm (Castagnaro et al., 1992; García-Olmedo et al., 1992). Type I corresponds to those thionins included in the original purothionin mixture obtained from wheat flour by Balls and co-workers (Balls et al., 1942). This mixture was resolved through CM-cellulose chromatography into two components, α and β purothionins (Redman and Fisher, 1968). The presence of structural genes for the α purothionin fraction in the long arms of chromosomes 1B and 1D of hexaploid wheat and for the β component in the long arm of chromosome 1A was subsequently demonstrated (Fernández de Caleya et al., 1976). The α purothionin fraction was later resolved by ion-exchange chromatography into two components, α_1 and α_2, whose amino acid sequences differ in six positions (Jones and Mak, 1977) and whose genes are respectively located on chromosomes 1B and 1D (Fernández de Caleya et al., 1976).

Here we report two cDNA clones, pTTH1 and pTTH14, respectively encoding the precursors of α_2 and α_1 purothionins (Table I). The deduced amino acid sequences for the mature protein domain are identical to those directly determined by Jones and Mak (1977). These clones were isolated under nonstringent conditions (58°C) from a cDNA library prepared from developing wheat endosperm, using a barley α-thionin cDNA probe (Ponz et al., 1986). Besides the mature thionin domain, the two precursors contain a signal peptide and a long C-terminal acidic protein, which is in line with the previously observed conservation of precursor structure across types (see García-Olmedo et al., 1992), even across extremely divergent types (Castagnaro et al., 1992), and suggests that all the precursors undergo similar co-translational and posttranslational processing steps (Ponz et al., 1983). Nucleotide sequences encoding the mature protein domain are more divergent (7.4% nucleotide substitutions) than the flanking sequences (signal peptide, 4%; acidic peptide, 3.5%; 3′ noncoding region, 2.5%) and the stop codon is three nucleotides downstream in the α_1 sequence.

Table I. *Characteristics of cDNAs encoding α_1 and α_2 thionins from hexaploid wheat*

Organism:
Triticum aestivum L. cv Chinese Spring.
Loci and Products:
Pur-B1, α_1 thionin; *Pur-D1*, α_2 thionin.
Relevant Feature of the Products:
Activity against plant pathogens both in vitro and in vivo.
Location in Genome:
Long arms of chromosomes 1B (α_1) and 1D (α_2) within a few kb of type-V thionin genes.
Techniques:
cDNA cloning and dideoxynucleotide sequencing of both DNA strands.
Method of Isolation and Subsequent Identification:
Clones isolated from a cDNA library of developing wheat endosperm by hybridization with the insert of barley thionin cDNA and pTH-1. Nucleotide sequences were compared to amino acid sequences of proteins.
Expression Characteristics:
Developmentally regulated, endosperm specific, synchronous with type V genes (8–25 d after pollination).
Features of cDNAs Structure:
Clones encode thionin precursors with three domains (signal peptide, mature protein, and C-terminal acidic protein).
Subcellular Localization:
Periphery of the protein bodies.
Antibodies:
Not available, but antibodies against barley type I thionins cross-react.

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The EMBL accession numbers for the sequences reported in this article are X770660 (pTTH1) and X70665 (pTTH14).

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